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(54) Title: SEED TREATMENT WITH COMBINATIONS OF PYRETHRINS/PYRETHROIDS AND THIAMETHOXAM

(57) Abstract: A method of preventing damage to the seed and/or shoots and foliage of a plant by a pest includes treating the seed from which the plant grows with a composition that includes a combination of thiamethoxam and at least one pyrethrin or synthetic pyrethroid which is selected from the group consisting of taufluvallinate, flumethrin, trans-cyfluthrin, kadethrin, bioresmethrin, tetramethrin, phenothrin, empenethrin, cyphenothrin, prallethrin, imiprothrin, allethrin and bioallethrin. The treatment is applied to the unsown seed. In another embodiment, the seed is a transgenic seed having at least one heterologous gene encoding for the expression of a protein having pesticidal activity against a first pest and the composition has activity against at least one second pest. Treated seeds are also provided.

**SEED TREATMENT WITH COMBINATIONS OF
PYRETHRINS/PYRETHROIDS AND THIAMETHOXAM**
CROSS REFERENCE TO RELATED PATENTS AND PATENT
APPLICATIONS

5 This application is a non-provisional of U.S. Provisional Pat.
Appl. No. 60/238,485, filed October 6, 2000, and claims priority thereto.

BACKGROUND OF THE INVENTION

(1) Field of the Invention

10 This invention relates generally to the control of plant pests and
more particularly to the provision of protection against insect damage
to seeds and plant parts by the treatment of plant seeds with
combinations of pesticides; in particular, the invention relates to the
control of insect damage to seeds and plant parts by the treatment of
plant seeds with a combination of thiamethoxam with pyrethrins and/or
15 synthetic pyrethroids.

(2) Description of the Related Art

20 The control of insects and related arthropods is of extreme
importance to the agricultural industry. Every year, these pests destroy
an estimated 15% of agricultural crops in the United States and even
more than that in developing countries. Some of this damage occurs in
the soil when plant pathogens, insects and other such soil borne pests
attack the seed after planting. Much of the rest of the damage is
caused by rootworms; plant pathogens that feed upon or otherwise
damage the plant roots; and by cutworms, European corn borers, and
25 other pests that feed upon or damage the above ground parts of the
plant. General descriptions of the type and mechanisms of attack of
pests on agricultural crops are provided by, for example, Metcalf, in
Destructive and Useful Insects, (1962); and Agrios, in *Plant Pathology*,
3rd Ed., Academic Press (1988).

30 The period during germination of the seed, sprouting and initial
growth of the plant is particularly critical because the roots and shoots
of the growing plant are small and even a small amount of damage can
kill the entire plant. Moreover, some natural plant defenses are not

fully developed at this stage and the plant is vulnerable to attack. Not surprisingly, the control of pests that attack the seed and the above ground plant parts during this early stage of plant growth is a well developed area of agriculture.

5 Currently, the control of pests that attack post emergent crops primarily involves the application of synthetic organic pesticides to the soil, or to the growing plants by foliar spraying. Because of concern about the impact of chemical pesticides on public health and the environment, there has been much effort to reduce the amount of
10 chemical pesticides that are used. A significant portion of this effort has been expended in developing transgenic crops engineered to express insect toxicants from microorganisms. For example, U.S. Patent No. 5,877,012 to Estruch *et al.* discloses the cloning and expression of proteins from such organisms as *Bacillus*,
15 *Pseudomonas*, *Clavibacter* and *Rhizobium* into plants to obtain transgenic plants with resistance to such pests as black cutworms, armyworms, several borers and other insect pests. Publication WO/EP97/07089 by Privalle *et al.* teaches the transformation of monocotyledons, such as corn, with a recombinant DNA sequence
20 encoding peroxidase for the protection of the plant from feeding by corn borers, earworms and cutworms. Jansens *et al.*, in *Crop Sci.*, 37(5):1616 - 1624 (1997), reported the production of transgenic corn containing a gene encoding a crystalline protein from *Bacillus*
 thuringiensis that controlled both generations of the European corn
25 borer. U. S. Patent Nos. 5,625,136 and 5,859,336 to Koziel *et al.* reported that the transformation of corn with a gene from *B. thuringiensis* that encoded for delta-endotoxins provided the transgenic corn with improved resistance to European corn borer.

 A comprehensive report of field trials of transgenic corn that
30 expresses an insecticidal protein from *B. thuringiensis* has been provided by Armstrong *et al.*, in *Crop Science*, 35(2):550 - 557 (1995).

 At the present state of plant cellular engineering, however, transgenic crops are typically resistant only to specific pests for that

crop, e.g., transgenic corn expressing a *Bt* toxin against the corn rootworm. It is frequently necessary to apply synthetic pesticides to such transgenic plants to control damage by other pests.

5 Insecticides such as synthetic pyrethroids, organophosphates and carbamates; fungicides such as azoles and anilopyrimidines; and acaricides such as pyrazoles; and the like, are very effective against certain above ground plant pests when applied at the proper time and with proper procedures. Appropriate pesticides may be applied at the time of planting as surface bands, "T"-bands, or in-furrow, but these
10 applications require the additional operation of applying the pesticide at the same time as the seeds are being sown. This complicates the planting operation and the additional equipment required for pesticide application is costly to purchase and requires maintenance and attention during use. Moreover, care must be taken to incorporate the
15 pesticides properly into the topmost soil layer for optimal activity. (See, for example, the application requirements and precautions for use of tefluthrin that are described in the brochure titled *Force 3G Insecticide*, published by Zeneca Ag Products, Wilmington, DE (1998)).

20 The activity of pesticides that have been applied as in-furrow applications at the time of sowing is usually directed to the protection of the seed or the roots of the plant. Some protection against above ground pests such as corn borers has been reported, however, for such treatments with insecticides known to be systemic. Keaster and Fairchild, *J. Econ. Entomol.*, 61(2):367 - 369 (1968). Since such
25 pesticide chemicals are complex molecules that are expensive to produce, purchase and use, it is desirable that their activity is not diluted or lost by migration away from the desired site of action by moisture seepage or by vaporization.

30 After the plant has emerged from the soil, foliar spraying of pesticides is most often used to control those pests that attach the shoots and foliage of the plant. However, a foliar spray must be applied at a certain time that coincides with the presence and activity of the pest in order to have the most beneficial effect. Application at this

time may be difficult or impossible if, for example, weather conditions limit access to the field. Moreover, the plants must be monitored closely to observe early signs of pest activity in order to apply the pesticide at a time when the pests are most vulnerable.

5 Synthetic pyrethroids have been found to give excellent control of pests of the order of Lepidoptera, such as cutworms, when applied as foliar spray or as surface-incorporated granules at the time of planting. However, since this class of insecticides has very high toxicity to fish, for example, great care must be taken to limit the runoff
10 of the insecticide from either granules or spray into surface waters. Moreover, any foliar spraying must be done at times when there is little wind, and then only with proper equipment that is carefully monitored during use.

 It has also been found in some cases with particular pesticides
15 and application techniques that when two or more of such pesticides are used in particular combination greater efficacy results than when any one of such pesticides is used alone. Such benefits of combining pesticides has been reported for combinations of phosmet with diflubenzuron (U.S. Patent No. 4,382,927); O-ethyl-O-[4-(methylthio)-
20 phenyl]-S-propyl phosphodithioate and N'-(4-chloro-o-tolyl)-N,N-dimethylformamidine (U.S. Patent No. 4,053,595); bacillus thuringiensis and chlordimeform (U.S. Patent No. 3,937,813); decamethrine and dichlorvos with propoxur, if desired, (U.S. Patent No. 4,863,909); fenvalerate and phosmet (U.S. Patent No. 4,263,287); and
25 phosalone and malathion (U.S. Patent No. 4,064,237). However, each of these combinations was applied directly to the growing plant as described above in the form of sprays or dusts, or applied to the soil around the plant in the form of, for example, granules.

 WO9740692 discloses combinations of any one of several
30 oxadiazine derivatives with one of a long list of other insecticides. Although the application mentions that the combinations can be applied to plant propagation material for its protection, as well as to plant shoots and leaves, no examples are provided to demonstrate that any

one of the combinations listed is actually efficacious. More pesticide combinations are described in U.S. Patent Nos. 4,415,561, 5,385,926, 5,972,941 and 5,952,358. However, in the existing art, little or no guidance has been found as methods for predicting which combinations of pesticides will result in such unexpectedly superior efficacy and which combinations will not.

The control of pests by applying insecticides directly to plant seed is well known. For example, U.S. Patent No. 5,696,144 discloses that the European corn borer caused less feeding damage to corn plants grown from seed treated with a 1-arylpyrazole compound at a rate of 500 g per quintal of seed than control plants grown from untreated seed. In addition, U.S. Patent No. 5,876,739 to Turnblad *et al.* (and its parent, U. S. Patent No. 5,849,320) disclose a method for controlling soil-borne insects which involves treating seeds with a coating containing one or more polymeric binders and an insecticide. This reference provides a list of insecticides that it identifies as candidates for use in this coating and also names a number of potential target insects. However, while the 5,876,739 patent states that treating corn seed with a coating containing a particular insecticide protects corn roots from damage by the corn rootworm, it does not indicate or otherwise suggest that treatment of corn seed with any particular combinations of insecticides provides the seed or the plant with synergistic protection, or with any other unexpected advantage.

Thus, although the art of protecting the shoots and foliage – as well as the seed and roots – of a plant from damage by pests has been advancing rapidly, several problems still remain. For example, it would be useful to provide a method for the control of pest damage to shoots and foliage of plants without the requirement of applying a pesticide at the time of sowing the seed, either as a surface incorporated band, or in-furrow, for example, or requiring a later field application of a pesticide during plant growth. It would also be useful if the method for pest control reduced the amount of pesticide that was required to provide a certain level of protection to the plant. Furthermore, it would

be useful if such a method could be coupled with the biopesticidal activity of transgenic plants, or with the insecticidal activity of other active materials to provide a broader scope of protection than is provided by the transgenic elements, or the insecticidal actives alone.

5 SUMMARY OF THE INVENTION

Briefly, therefore, the present invention is directed to a novel method for preventing damage by a pest to a seed and/or shoots and foliage of a plant grown from the seed, the method comprising treating the unsown seed with a composition comprising thiamethoxam and at least one pyrethrin or synthetic pyrethroid. It is preferred that the pyrethrin or pyrethroid is one or more selected from the group consisting of taufluvalinate, flumethrin, trans-cyfluthrin, kadethrin, bioresmethrin, tetramethrin, phenothrin, empenethrin, cyphenothrin, prallethrin, imiprothrin, allethrin and bioallethrin. Seeds that have been treated by this method are also provided.

The invention is also directed to a novel composition for the treatment of unsown seed comprising thiamethoxam and at least one pyrethrin or synthetic pyrethroid. Here again, it is preferred that the pyrethrin or synthetic pyrethroid is one or more selected from the group consisting of taufluvalinate, flumethrin, trans-cyfluthrin, kadethrin, bioresmethrin, tetramethrin, phenothrin, empenethrin, cyphenothrin, prallethrin, imiprothrin, allethrin and bioallethrin.

The invention is also directed to a novel seed that is protected against multiple pests comprising a seed having at least one heterologous gene encoding for the expression of a protein that is active against a first pest and, in addition, having adhered thereto a composition comprising thiamethoxam and at least one pyrethrin or synthetic pyrethroid, where the composition is present in an amount effective to provide protection to the shoots and foliage of the plant against damage by at least one second pest.

The invention is also directed to a novel method for treating an unsown seed to prevent damage by a pest to the seed and/or shoots and foliage of a plant grown from the seed, the method comprising

contacting the unsown seed with a composition comprising thiamethoxam and at least one pyrethrin or synthetic pyrethroid selected from the group consisting of taufluvalinate, flumethrin, trans-cyfluthrin, kadethrin, bioresmethrin, tetramethrin, phenothrin, 5 empenethrin, cyphenothrin, prallethrin, imiprothrin, allethrin and bioallethrin.

Among the advantages found to be achieved by the present invention, therefore, may be noted the provision of a method for the control of pest damage to seeds and/or shoots and foliage of plants 10 without the requirement of applying a pesticide at the time of sowing the seed, either as a surface incorporated band, or in-furrow, for example, or requiring a later field application of a pesticide during plant growth; the provision of a method for pest control that reduces the amount of pesticide that is required for the provision of a certain level 15 of protection to the plant; and the provision of method that can be coupled with the biopesticidal activity of transgenic plants to selectively broaden the scope of protection that is provided for the shoots and foliage of the transgenic plant.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

20 In accordance with the present invention, it has been discovered that treatment of unsown plant seeds with a composition that includes a specific combination of insecticides not only protects the seeds themselves, but – surprisingly – also provides post-emergent control of pests that feed on or otherwise damage the shoots and/or foliage of the 25 plant. The combination of insecticides that has been found to achieve such results is a combination of a pyrethrin or synthetic pyrethroid and thiamethoxam.

In preferred embodiments, the subject combination of insecticides provides unexpectedly superior protection in that the 30 combination of the insecticides provides a level of protection to the seed and/or the plant that is superior to the level of protection that – based on the current state of the art – would be predicted from the protection provided by the individual components applied separately.

This synergistic activity reduces the total amount of pesticide that is required to provide a certain level of protection. In addition to being more economical to use, the ability to use a reduced amount of pesticide for a given level of protection is advantageous in that seed treatments with reduced amounts of insecticides are less phytotoxic to the seed than when the insecticides are used separately.

Another advantage of the novel treatment is that it can be used with transgenic seeds of the type having a heterologous gene encoding for the expression of a pesticidal protein in the transgenic plant that grows from the seed. Treating such a seed with a pesticide provides the ability to protect against one pest with the transgenic trait and to provide surprisingly enhanced protection against the same pest, and/or to protect against other pests with the subject combination of insecticides.

As used herein, the terms "pesticidal effect" and "pesticidal activity" mean any direct or indirect action on the target pest that results in reduced feeding damage on the seeds, roots, shoots and foliage of plants grown from treated seeds as compared to plants grown from untreated seeds. The terms "active against a (first or second) pest", also have the same meaning. Such direct or indirect actions include inducing death of the pest, repelling the pest from the plant seeds, roots, shoots and/or foliage, inhibiting feeding of the pest on, or the laying of its eggs on, the plant seeds, roots, shoots and/or foliage, and inhibiting or preventing reproduction of the pest. The term "insecticidal activity" has the same meaning as pesticidal activity, except it is limited to those instances where the pest is an insect. When the term "pesticide" is used herein, it is not meant to include pesticides that are produced by the particular seed or the plant that grows from the particular seed that is treated with the pesticide.

As used herein, the "shoots and foliage" of a plant are to be understood to be the shoots, stems, branches, leaves and other appendages of the stems and branches of the plant after the seed has sprouted, but not including the roots of the plant. It is preferable that

the shoots and foliage of a plant be understood to be those non-root parts of the plant that have grown from the seed and are located a distance of at least one inch away from the seed from which they emerged (outside the region of the seed), and more preferably, to be the non-root parts of the plant that are at or above the surface of the soil. As used herein, the "region of the seed" is to be understood to be that region within about one inch of the seed.

Pesticides suitable for use in the invention include pyrethrins and synthetic pyrethroids and thiamethoxam. Information about pyrethrins and pyrethroids and thiamethoxam can be found in *The Pesticide Manual*, 11th Ed., C. D. S. Tomlin, Ed., British Crop Protection Council, Farnham, Surrey, UK (1997).

Pyrethroids that are useful in the present composition include pyrethrins and synthetic pyrethroids. The pyrethrins that are preferred for use in the present method include, without limitation, 2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one ester of 2,2-dimethyl-3-(2methylpropenyl)-cyclopropane carboxylic acid, and/or (2-methyl-1-propenyl)-2-methoxy-4-oxo-3-(2 propenyl)-2-cyclopenten-1-yl ester and mixtures of cis and trans isomers thereof (Chemical Abstracts Service Registry Number ("CAS RN") 8003-34-7).

Synthetic pyrethroids that are preferred for use in the present invention include (s)-cyano(3-phenoxyphenyl)methyl 4-chloro alpha (1-methylethyl)benzeneacetate (fenvalerate; CAS RN 51630-58-1); (S)-cyano (3-phenoxyphenyl) methyl (S)-4-chloro-alpha-(1-methylethyl)benzeneacetate (esfenvalerate; CAS RN 66230-04-4); (3-phenoxyphenyl)-methyl(+)cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate (permethrin; CAS RN 52645-53-1); (±) alpha-cyano-(3-phenoxyphenyl) methyl(+)-cis,trans-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylate (cypermethrin; CAS RN 52315-07-8); (beta-cypermethrin; CAS RN 65731-84-2); (theta cypermethrin; CAS RN 71697-59-1); S-cyano (3-phenoxyphenyl) methyl (±) cis/trans 3-(2,2-dichloroethenyl) 2,2 dimethylcyclopropane carboxylate (zeta-cypermethrin; CAS RN 52315-07-8); (s)-alpha-

cyano-3-phenoxybenzyl (IR,3R)-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropanecarboxylate (deltamethrin; CAS RN 52918-63-5); alpha-cyano-3-phenoxybenzyl 2,2,3,3,-tetramethyl cyclopropoanecarboxylate (fenpropathrin; CAS RN 64257-84-7); (RS)-alpha-cyano-3-
5 phenoxybenzyl(R)-2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoate (tau-fluvalinate; CAS RN 102851-06-9); (2,3,5,6-tetrafluoro-4-methylphenyl)-methyl-(1 alpha, 3 alpha)-(Z)-(±)-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate (tefluthrin; CAS RN 79538-32-2); (±)-cyano (3-phenoxyphenyl) methyl
10 (±)-4-(difluoromethoxy)-alpha-(1-methyl ethyl) benzeneacetate (flucythrinate; CAS RN 70124-77-5); cyano(4-fluoro-3-phenoxyphenyl)methyl 3-[2-chloro-2-(4-chlorophenyl)ethenyl]-2,2-dimethylcyclopropanecarboxylate (flumethrin; CAS RN 69770-45-2);
15 cyano(4-fluoro-3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropanedarboxylate (cyfluthrin; CAS RN 68359-37-5); (beta cyfluthrin; CAS RN 68359-37-5); (transfluthrin; CAS RN 118712-89-3); (S)-alpha-cyano-3-phenoxybenzyl(Z)-(IR-cis)-2,2-dimethyl-3-[2-(2,2,2-trifluoro-trifluoromethyl-ethoxycarbonyl)vinyl]cyclopropane carboxylate (acrinathrin; CAS RN 101007-06-1); (IR cis) S and (IS cis)
20 R enantiomer isomer pair of alpha-cyano-3-phenoxybenzyl-3-(2,2dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (alpha-cypermethrin; CAS RN 67375-30-8); [IR,3S)3(1'RS)(1',2',2',2'-tetrabromoethyl)]-2,2-dimethyl cyclopropanecarboxylic acid (s)-alpha-cyano-3-phenoxybenzyl ester (tralomethrin; CAS RN 66841-25-6);
25 cyano-(3-phenoxyphenyl) methyl 2,2-dichloro-1- (4-ethoxyphenyl)cyclopropane carboxylate (cycloprothrin; CAS RN 63935-38-6); [1α, 3α(Z)]-(±)-cyano-(3-phenoxyphenyl)methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-cimethylcyclopropanecarboxylate (cyhalothrin; CAS RN 68085-85-8); [1 alpha (s), 3 alpha(z)]-cyano(3-phenoxyphenyl) methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-
30 dimethylcyclopropane carboxylate (lambda cyhalothrin; CAS RN 91465-08-6); (2-methyl [1,1'-biphenyl]-3-yl) methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyclopropanecarboxylate (bifenthrin;

CAS RN 82657-04-3); 5-1-benzyl-3-furymethyl-d-cis(1R,3S,E)2,2-dimethyl-3-(2-oxo,-2,2,4,5 tetrahydro thiophenylidenemethyl)cyclopropane carboxylate (kadethrin, RU15525; CAS RN 58769-20-3); [5-(phenyl methyl)-3-furanyl]-3-furanyl 2,2-dimethyl-3-(2-methyl-1-propenyl) cyclopropane carboxylate (resmethrin; CAS RN 10453-86-8); (1R-trans)-[5-(phenylmethyl)-3-furanyl]methyl 2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate (bioresmethrin; CAS RN 28434-01-7); 3,4,5,6-tetra hydro-phthalimidomethyl-(IRS)-cis-trans-chrysanthemate (tetramethrin; CAS RN 7696-12-0); 3-phenoxybenzyl-d,l-cis,trans 2,2-dimethyl-3-(2-methylpropenyl) cyclopropane carboxylate (phenothrin; CAS RN 26002-80-2); (empenthrin; CAS RN 54406-48-3); (cyphenothrin; CAS RN 39515-40-7); (prallethrin; CAS RN 23031-36-9); (imiprothrin; CAS RN 72963-72-5); (RS)-3-allyl-2-methyl-4-oxocyclopent-2-enyl-(1A,3R; 1R,3S)-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate (allethrin; CAS RN 584-79-2); (bioallethrin; CAS RN 584-79-2); and (ZXI8901; CAS RN 160791-64-0). It is believed that mixtures of one or more of the aforementioned synthetic pyrethroids can also be used in the present invention.

In one embodiment of the present invention, it is preferred that the pyrethrin or synthetic pyrethroid is limited to a pyrethroid selected from the group consisting of taufluvalinate, flumethrin, trans-cyfluthrin, kadethrin, bioresmethrin, tetramethrin, phenothrin, empenthrin, cyphenothrin, prallethrin, imiprothrin, allethrin and bioallethrin.

The pyrethrins and synthetic pyrethroids that are useful in the present compositions can be of any grade or purity that pass in the trade as pyrethrins and synthetic pyrethroids. Other materials that accompany the pyrethrins and synthetic pyrethroids in commercial preparations as impurities can be tolerated in the subject compositions, as long as such other materials do not destabilize the composition or significantly reduce or destroy the activity of any of the insecticide components against the target pest. One of ordinary skill in the art of

the production of insecticides can readily identify those impurities that can be tolerated and those that cannot.

Thiamethoxam (3-[(2-chloro-5-thiazoyl)methyl]tetrahydro-5-methyl-N-nitro-4H-1,3,5-oxadiazin-4-imine; CAS RN 153719-23-4) comprises one of the components of insecticides of the present combination.

The thiamethoxam insecticides that are useful in the present compositions can be of any grade or purity that pass in the trade as thiamethoxam. Other materials that accompany the thiamethoxam in commercial preparations as impurities can be tolerated in the subject compositions, as long as such other materials do not destabilize the composition or significantly reduce or destroy the activity of any of the insecticide components against the target pest. One of ordinary skill in the art of the production of insecticides can readily identify those impurities that can be tolerated and those that cannot.

When an insecticide is described herein, it is to be understood that the description is intended to include salt forms of the insecticide as well as any isomeric and/or tautomeric form of the insecticide that exhibits the same insecticidal activity as the form of the insecticide that is described.

One embodiment of this invention comprises treating a seed with a composition comprising at least one pyrethrin or synthetic pyrethroid and thiamethoxam. The treatment is applied to the seed prior to sowing the seed so that the sowing operation is simplified. In this manner, seeds can be treated, for example, at a central location and then dispersed for planting. This permits the person who plants the seeds to avoid the handling and use of insecticides -- some of which can be toxic -- and to merely handle and plant the treated seeds in a manner that is conventional for regular untreated seeds. It is preferred in some combinations that at least one of the pyrethrins or synthetic pyrethroids is a systemic insecticide.

In one embodiment of the present method, a seed can be treated with a combination of thiamethoxam and any one of the

insecticides that are shown in Table 1. In fact, two or more of the pesticides listed in Table 1 can be used in combination with thiamethoxam.

- 5 Table 1: Pesticides that can be used in combination with thiamethoxam that are believed to provide synergistic insecticidal activity^a.

<u>COMPOSITION</u>	<u>PYRETHROID</u>
<u>NO.</u>	
1	lambda-cyhalothrin
2	tefluthrin
3	cyfluthrin
4	bifenthrin
5	fenvalerate
6	esfenvalerate
7	permethrin
8	cypermethrin
9	beta-cypermethrin
10	zeta-cypermethrin
11	deltamethrin
12	fenpropathrin
13	tafluvalinate
14	flucythrinate
15	flumethrin
16	beta-cyfluthrin
17	trans-cyfluthrin
18	acrinathrin
19	alphacypermethrin
20	tralomethrin
21	cycloprothrin
22	kadethrin
23	resmethrin
24	bioresmethrin
25	tetramethrin
26	phenothrin
27	empenthrin
28	cyphenothrin
29	prallethrin
30	imiprothrin
31	allethrin
32	bioallethrin

Note:

- 10 a. The composition comprises thiamethoxam and the insecticide that appears on the same line as the number of the composition.

When thiamethoxam is present in the subject combination, it has been found that it is preferred that the at least one pyrethroid be selected from the group consisting of taufluvalinate, flumethrin, trans-
5 cyfluthrin, kadethrin, bioresmethrin, tetramethrin, phenothrin, empenethrin, cyphenothrin, prallethrin, imiprothrin, allethrin and bioallethrin. Preferred combinations include thiamethoxam and taufluvalinate, thiamethoxam and flumethrin, thiamethoxam and trans-
10 cyfluthrin, thiamethoxam and kadethrin, thiamethoxam and bioresmethrin, thiamethoxam and tetramethrin, thiamethoxam and phenothrin, thiamethoxam and empenethrin, thiamethoxam and cyphenothrin, thiamethoxam and prallethrin, thiamethoxam and imiprothrin, thiamethoxam and allethrin and thiamethoxam and bioallethrin.

15 It has also been found that a transgenic seed can be protected against multiple pests when the seed has at least one heterologous gene encoding for the expression of a protein that is active against a first pest and, in addition, having adhered thereto a composition comprising at least one pyrethrin or synthetic pyrethroid and
20 thiamethoxam. It is preferred that the composition containing the synergistic combination of insecticides is present in an amount effective to provide protection to the shoots and foliage of the plant against damage by at least one second pest. It is more preferred that the composition is present in an amount effective to provide a
25 synergistic effect.

When the transgenic seed has at least one heterologous gene encoding for the expression of a protein that is active against a first pest, the seed can be treated with a synergistic combination of insecticides, which combination has activity against at least one
30 second pest. The present method can be used when the first pest and the second pest are the same, for the purpose, for example, to obtain effective control of a particularly resistant or highly damaging pest. But in a separate embodiment, the transgenic trait protects the seed and/or

plant from a first pest and the composition of the combination of insecticides is selected to control a second pest that is different from the first pest. This method is particularly advantageous when an expressed transgenic gene provides a gene product that can protect a transgenic plant from one pest, but has no activity against a second, different pest. In this case, a combination of insecticides of the present invention can be selected that has activity against the second pest, thus providing the seed and plant with protection from both pests. By way of explanation, when a "first" pest and a "second" pest are referred to herein, it should be understood that each of the terms can include only one pest, or can include two or more pests.

It is contemplated that the present method can be used to protect the seeds, roots and/or the above-ground parts of field, forage, plantation, glasshouse, orchard or vineyard crops, ornamentals, plantation or forest trees. The seeds that are useful in the present invention can be the seeds of any species of plant. However, they are preferably the seeds of plant species that are agronomically important. In particular, the seeds can be of corn, peanut, canola/rapeseed, soybean, cucurbits, crucifers, cotton, beets, rice, sorghum, sugar beet, wheat, barley, rye, sunflower, tomato, sugarcane, tobacco, oats, as well as other vegetable and leaf crops. It is preferred that the seed be corn, soybeans, or cotton seed; and more preferred that the seeds be corn seeds.

In one embodiment of the invention, as mentioned above, the seed is a transgenic seed from which a transgenic plant can grow. The transgenic seed of the present invention is engineered to express a desirable characteristic and, in particular, to have at least one heterologous gene encoding for the expression of a protein that is pesticidally active and, in particular, has insecticidal activity. The heterologous gene in the transgenic seeds of the present invention can be derived from a microorganism such as *Bacillus*, *Rhizobium*, *Pseudomonas*, *Serratia*, *Trichoderma*, *Clavibacter*, *Glomus*, *Gliocladium* and mycorrhizal fungi. In particular, it is believed that the

present method would be especially beneficial when the heterologous gene is one that is derived from a *Bacillus sp.* microorganism and the protein is active against corn rootworm. It is also believed that the present method would be especially beneficial when the heterologous gene is one that is derived from a *Bacillus sp.* microorganism and the protein is active against European corn borer. A preferred *Bacillus sp.* microorganism is *Bacillus thuringiensis*. It is particularly preferred when the heterologous gene encodes a modified Cry3Bb delta-endotoxin derived from *Bacillus thuringiensis*.

The target pest for the present invention is an adult or larvae of any insect or other pest that feeds on the seed, roots and/or shoots and foliage of the plant that is to be protected by the subject method. Such pests include but are not limited to: from the order Lepidoptera, for example,

Acleris spp., *Adoxophyes* spp., *Aegeria* spp., *Agrotis* spp., *Alabama argillaceae*, *Amylois* spp., *Anticarsia gemmatilis*, *Archips* spp., *Argyrotaenia* spp., *Autographa* spp., *Busseola fusca*, *Cadra cautella*, *Carposina nipponensis*, *Chilo* spp., *Choristoneura* spp., *Clysia ambiguella*, *Cnaphalocrocis* spp., *Cnephasia* spp., *Cochylis* spp., *Coleophora* spp., *Crocidolomia binotalis*, *Cryptophlebia leucotreta*, *Cydia* spp., *Diatraea* spp., *Diparopsis castanea*, *Earias* spp., *Ephestia* spp., *Eucosma* spp., *Eupoecilia ambiguella*, *Euproctis* spp., *Euxoa* spp., *Grapholita* spp., *Hedya nubiferana*, *Heliothis* spp., *Hellula undalis*, *Hyphantria cunea*, *Keiferia lycopersicella*, *Leucoptera scitella*, *Lithocollethis* spp., *Lobesia botrana*, *Lymantria* spp., *Lyonetia* spp., *Malacosoma* spp., *Mamestra brassicae*, *Manduca sexta*, *Operophtera* spp., *Ostrinia Nubilalis*, *Pammene* spp., *Pandemis* spp., *Panolis flammea*, *Pectinophora gossypiella*, *Phthorimaea operculella*, *Pieris rapae*, *Pieris* spp., *Plutella xylostella*, *Prays* spp., *Scirpophaga* spp., *Sesamia* spp., *Sparganothis* spp., *Spodoptera* spp., *Synanthedon* spp., *Thaumatopoea* spp., *Tortrix* spp., *Trichoplusia ni* and *Yponomeuta* spp.; from the order Coleoptera, for example,

- Agriotes spp., Anthonomus spp., *Atomaria linearis*,
Chaetocnema tibialis, Cosmopolites spp., Curculio spp., Dermestes
spp., Diabrotica spp., Epilachna spp., Eremnus spp., *Leptinotarsa*
decemlineata, Lissorhoptrus spp., Melolontha spp., Oryzaephilus spp.,
5 Otiorhynchus spp., Phlyctinus spp., Popillia spp., Psylliodes spp.,
Rhizopertha spp., Scarabeidae, Sitophilus spp., Sitotroga spp.,
Tenebrio spp., Tribolium spp. and Trogoderma spp.;
from the order Orthoptera, for example,
Blatta spp., Blattella spp., Gryllotalpa spp., *Leucophaea*
10 *maderae*, Locusta spp., Periplaneta spp., and Schistocerca spp.;
from the order Isoptera, for example,
Reticulitermes spp.;
from the order Psocoptera, for example,
Liposcelis spp.;
15 from the order Anoplura, for example,
Haematopinus spp., Linognathus spp., Pediculus spp.,
Pemphigus spp. and Phylloxera spp.;
from the order Mallophaga, for example,
Damalinae spp. and Trichodectes spp.;
20 from the order Thysanoptera, for example,
Franklinella spp., Hercinothrips spp., Taeniothrips spp., *Thrips*
palmi, *Thrips tabaci* and *Scirtothrips aurantii*;
from the order Heteroptera, for example,
Cimex spp., *Distantiella theobroma*, Dysdercus spp., Euchistus
25 spp., Eurygaster spp., Leptocorisa spp., Nezara spp., Piesma spp.,
Rhodnius spp., *Sahlbergella singularis*, Scotinophara spp. and
Triatoma spp.;
from the order Homoptera, for example,
Aleurothrixus floccosus, *Aleyrodes brassicae*, Aonidiella spp.,
30 Aphididae, Aphis spp., Aspidiotus spp., *Bemisia tabaci*, Ceroplaster
spp., *Chrysomphalus aonidium*, *Chrysomphalus dictyospermi*, *Coccus*
hesperidum, Empoasca spp., *Eriosoma larigerum*, Erythroneura spp.,
Gascardia spp., Laodelphax spp., *Lacanium corni*, Lepidosaphes spp.,

Macrosiphus spp., Myzus spp., Nehotettix spp., Nilaparvata spp.,
Paratoria spp., Pemphigus spp., Planococcus spp., Pseudaulacaspis
spp., Pseudococcus spp., Psylla spp., *Pulvinaria aethiopica*,
Quadraspidotus spp., Rhopalosiphum spp., Saissetia spp.,
5 Scaphoideus spp., Schizaphis spp., Sitobion spp., *Trialeurodes*
vaporariorum, *Trioza erythrae* and *Unaspis citri*;
from the order Hymenoptera, for example,

Acromyrmex, Atta spp., Cephus spp., Diprion spp., Diprionidae,
Gilpinia polytoma, Hoplocampa spp., Lasius spp., *Monomorium*
10 *pharaonis*, Neodiprion spp., Solenopsis spp. and Vespa spp.;
from the order Diptera, for example,

Aedes spp., *Antherigona soccata*, *Bibio hortulanus*, *Calliphora*
erythrocephala, Ceratitis spp., Chrysomyia spp., Culex spp., Cuterebra
spp., Dacus spp., *Drosophila melanogaster*, Fannia spp., Gastrophilus
15 spp., Glossina spp., Hypoderma spp., Hyppobosca spp., Liriomyza
spp., Lucilia spp., Melanagromyza spp., Musca spp., Oestrus spp.,
Orseolia spp., *Oscinella frit*, *Pegomyia hyoscyami*, Phorbia spp.,
Rhagoletis pomonella, Sciara spp., Stomoxys spp., Tabanus spp.,
Tannia spp. and Tipula spp.,

20 from the order Siphonaptera, for example,

Ceratophyllus spp. und *Xenopsylla cheopis* and
from the order Thysanura, for example,

Lepisma saccharina.

In each embodiment of the invention, a combination of two or
25 more insecticides is applied to a seed in an effective amount; that is, an
amount sufficient to provide protection to the seed and/or shoots and
foliage of the plant that grows from the seed. As used herein,
"protection" is achieved if the percent of feeding damage to the seed
and/or the shoots and foliage at 10 days after infestation (DAI) with the
30 pest is reduced for treated seeds or plants grown from treated seeds
as compared to untreated seeds or plants grown from untreated seeds.
In a preferred embodiment, an unexpected advantage of the
compositions of the present invention is that the component

insecticides of the composition operate synergistically. As used here, when it is said that a combination demonstrates "synergy", what is meant is that the degree of protection that is provided to a seed and/or the shoots and foliage of a plant that grows from a seed, by treatment of the seed by the present method (using a combination of insecticides), is superior to the degree of protection that would be expected on the basis of the protection provided by each of the components of the composition applied separately.

Methods for the calculation of whether a particular insecticide combination provides a synergistic degree of protection against pests are described in detail in the Examples. Briefly stated, however, whether a combination of insecticides provided synergy in protection against cutworm damage can be calculated as described by Colby, Robert. S., in *Weeds*, 15(1):20 - 22 (1967). The threshold value (stated as % of control) for synergy of a combination was calculated as $= (\% \text{ of control for treatment A}) * (\% \text{ of control for treatment B}) / 100(n-1)$; where n = number of active ingredients in the combination. A measured % of control value that is less than the calculated threshold value indicates synergy of the combination.

When the "degree of protection" is mentioned herein, it is meant to include the amount of damage caused by the target insect to seeds that have been treated with a given amount of insecticide (and the plants that sprout therefrom) relative to the amount of damage caused to untreated seeds and plants. But "degree of protection" can also refer to the number of different types of target pests that are affected by the treatment and the length of the period of protection. In other words, a synergistic degree of protection can include unexpectedly effective protection at reduced levels of active ingredient, as well as protection against an unexpectedly wide variety of pests, or protection for an unexpectedly long (or otherwise particularly effective) period of time.

The amount of the insecticidal composition of the present invention that will provide protection to plant shoots and foliage will

vary depending on the particular pesticide combination, the concentration of active ingredients in the composition, the nature of the formulation in which it is applied, the seed type, and the target pest(s). As used herein, an amount of the composition effective to provide protection to the seed and/or shoots and foliage of the plant against damage by the pest is the lowest amount of such pesticide that will provide such protection. Assuming that the composition is comprised of 100% active ingredients, then, in general, the amount of the subject composition used will range from about 0.005% to 25% of the weight of the seed, and more preferably, from about 0.01% to about 10%. A yet more preferred range is 0.01% to 1% of the active ingredients relative to the weight of the seed, and an even more preferred range is 0.05% to 0.5%.

The subject compositions are each composed of at least two insecticidal compounds, such as the combinations described in Table 1, and in the surrounding text. When two components are used, the relative amounts of the two insecticides can range from 1:1000 to 1000:1, by weight. It is preferred, however, that the weight ratio of the two insecticides range from 1:100 to 100:1, more preferred is a ratio of 1:10 to 10:1, and yet more preferred is a ratio of 1:3 to 3:1.

In the method of the present invention, the combination of pesticides is applied to a seed. Although it is believed that the present method can be applied to a seed in any physiological state, it is preferred that the seed be in a sufficiently durable state that it incurs no damage during the treatment process. Typically, the seed would be a seed that had been harvested from the field; removed from the plant; and separated from any cob, stalk, outer husk, and surrounding pulp or other non-seed plant material. The seed would preferably also be biologically stable to the extent that the treatment would cause no biological damage to the seed. In one embodiment, for example, the treatment can be applied to seed corn that has been harvested, cleaned and dried to a moisture content below about 15% by weight. In an alternative embodiment, the seed can be one that has been dried

and then primed with water and/or another material and then re-dried before or during the treatment with the pesticide. Within the limitations just described, it is believed that the treatment can be applied to the seed at any time between harvest of the seed and sowing of the seed.

5 As used herein, the term "unsown seed" is meant to include seed at any period between the harvest of the seed and the sowing of the seed in the ground for the purpose of germination and growth of the plant.

When it is said that unsown seed is "treated" with the composition, such treatment is not meant to include those practices in which the pesticide is applied to the soil, rather than to the seed. For example, such treatments as the application of the pesticide in bands, "T"-bands, or in-furrow, at the same time as the seed is sowed are not considered to be included in the present invention.

15 The composition comprising a combination of pesticides, can be applied "neat", that is, without any diluting or additional components present. However, the composition is typically applied to the seeds in the form of a pesticide formulation. This formulation may contain one or more other desirable components including but not limited to liquid diluents, binders to serve as a matrix for the pesticide, fillers for protecting the seeds during stress conditions, and plasticizers to improve flexibility, adhesion and/or spreadability of the coating. In addition, for oily pesticide formulations containing little or no filler, it may be desirable to add to the formulation drying agents such as calcium carbonate, kaolin or bentonite clay, perlite, diatomaceous earth
20 or any other adsorbent material. Use of such components in seed treatments is known in the art. See, *e.g.*, U.S. Patent No. 5,876,739. The skilled artisan can readily select desirable components to use in the pesticide formulation depending on the seed type to be treated and the particular pesticide that is selected. In addition, readily available
25 commercial formulations of known pesticides may be used, as demonstrated in the examples below.

30 The seeds may also be treated with one or more of the following ingredients: other pesticides, including compounds which act only

below the ground; fungicides, such as captan, thiram, metalxyl, fludioxonil, oxadixyl, and isomers of each of those materials, and the like; herbicides, including compounds selected from carbamates, thiocarbamates, acetamides, triazines, dinitroanilines, glycerol ethers, pyridazinones, uracils, phenoxys, ureas, and benzoic acids; herbicidal safeners such as benzoxazine, benzhydryl derivatives, N,N-diallyl dichloroacetamide, various dihaloacyl, oxazolidinyl and thiazolidinyl compounds, ethanone, naphthalic anhydride compounds, and oxime derivatives; fertilizers; and biocontrol agents such as naturally-occurring or recombinant bacteria and fungi from the genera *Rhizobium*, *Bacillus*, *Pseudomonas*, *Serratia*, *Trichoderma*, *Glomus*, *Gliocladium* and mycorrhizal fungi. These ingredients may be added as a separate layer on the seed or alternatively may be added as part of the pesticide composition.

Preferably, the amount of the novel composition or other ingredients used in the seed treatment should not inhibit generation of the seed, or cause phytotoxic damage to the seed.

The composition of the present invention can be in the form of a suspension; emulsion; slurry of particles in an aqueous medium (e.g., water); wettable powder; wettable granules (dry flowable); and dry granules. If formulated as a suspension or slurry, the concentration of the active ingredient in the formulation is preferably about 0.5% to about 99% by weight (w/w), preferably 5-40%.

As mentioned above, other conventional inactive or inert ingredients can be incorporated into the formulation. Such inert ingredients include but are not limited to: conventional sticking agents, dispersing agents such as methylcellulose (Methocel A15LV or Methocel A15C, for example, serve as combined dispersant/sticking agents for use in seed treatments), polyvinyl alcohol (e.g., Elvanol 51-05), lecithin (e.g., Yelkinol P), polymeric dispersants (e.g., polyvinylpyrrolidone/vinyl acetate PVP/VA S-630), thickeners (e.g., clay thickeners such as Van Gel B to improve viscosity and reduce settling of particle suspensions), emulsion stabilizers, surfactants, antifreeze

compounds (e.g., urea), dyes, colorants, and the like. Further inert ingredients useful in the present invention can be found in McCutcheon's, vol. 1, *"Emulsifiers and Detergents,"* MC Publishing Company, Glen Rock, New Jersey, U.S.A., 1996. Additional inert
5 ingredients useful in the present invention can be found in McCutcheon's, vol. 2, *"Functional Materials,"* MC Publishing Company, Glen Rock, New Jersey, U.S.A., 1996.

The pesticides, compositions of pesticide combinations, and formulations of the present invention can be applied to seeds by any
10 standard seed treatment methodology, including but not limited to mixing in a container (e.g., a bottle or bag), mechanical application, tumbling, spraying, and immersion. Any conventional active or inert material can be used for contacting seeds with pesticides according to the present invention, such as conventional film-coating materials
15 including but not limited to water-based film coating materials such as Sepiret (Seppic, Inc., Fairfield, NJ) and Opacoat (Berwind Pharm. Services, Westpoint, PA).

The subject combination of pesticides can be applied to a seed as a component of a seed coating. Seed coating methods and
20 compositions that are known in the art are useful when they are modified by the addition of one of the embodiments of the combination of pesticides of the present invention. Such coating methods and apparatus for their application are disclosed in, for example, U.S. Patent Nos. 5,918,413, 5,891,246, 5,554,445, 5,389,399, 5,107,787,
25 5,080,925, 4,759,945 and 4,465,017. Seed coating compositions are disclosed, for example, in U.S. Patent Nos. 5,939,356, 5,882,713, 5,876,739, 5,849,320, 5,834,447, 5,791,084, 5,661,103, 5,622,003, 5,580,544, 5,328,942, 5,300,127, 4,735,015, 4,634,587, 4,383,391, 4,372,080, 4,339,456, 4,272,417 and 4,245,432, among others.

30 Useful seed coatings contain one or more binders and at least one of the subject combinations of pesticides.

Binders that are useful in the present invention preferably comprise an adhesive polymer that may be natural or synthetic and is

without phytotoxic effect on the seed to be coated. The binder may be selected from polyvinyl acetates; polyvinyl acetate copolymers; polyvinyl alcohols; polyvinyl alcohol copolymers; celluloses, including ethylcelluloses, methylcelluloses, hydroxymethylcelluloses, hydroxypropylcelluloses and carboxymethylcellulose; polyvinylpyrrolidones; polysaccharides, including starch, modified starch, dextrans, maltodextrins, alginate and chitosans; fats; oils; proteins, including gelatin and zeins; gum arabics; shellacs; vinylidene chloride and vinylidene chloride copolymers; calcium lignosulfonates; acrylic copolymers; polyvinylacrylates; polyethylene oxide; acrylamide polymers and copolymers; polyhydroxyethyl acrylate, methylacrylamide monomers; and polychloroprene.

It is preferred that the binder be selected so that it can serve as a matrix for the subject combination of pesticides. While the binders disclosed above may all be useful as a matrix, the specific binder will depend upon the properties of the combination of pesticides. The term "matrix", as used herein, means a continuous solid phase of one or more binder compounds throughout which is distributed as a discontinuous phase one or more of the subject combinations of pesticides. Optionally, a filler and/or other components can also be present in the matrix. The term matrix is to be understood to include what may be viewed as a matrix system, a reservoir system or a microencapsulated system. In general, a matrix system consists of a combination of pesticides of the present invention and filler uniformly dispersed within a polymer, while a reservoir system consists of a separate phase comprising the subject combination of pesticides, that is physically dispersed within a surrounding, rate-limiting, polymeric phase. Microencapsulation includes the coating of small particles or droplets of liquid, but also to dispersions in a solid matrix.

The amount of binder in the coating can vary, but will be in the range of about 0.01 to about 25% of the weight of the seed, more preferably from about 0.05 to about 15%, and even more preferably from about 0.1% to about 10%.

As mentioned above, the matrix can optionally include a filler.

The filler can be an absorbent or an inert filler, such as are known in the art, and may include woodflours, clays, activated carbon, sugars, diatomaceous earth, cereal flours, fine-grain inorganic solids, calcium carbonate, and the like. Clays and inorganic solids which may be used include calcium bentonite, kaolin, china clay, talc, perlite, mica, vermiculite, silicas, quartz powder, montmorillonite and mixtures thereof. Sugars which may be useful include dextrin and maltodextrin. Cereal flours include wheat flour, oat flour and barley flour.

The filler is selected so that it will provide a proper microclimate for the seed, for example the filler is used to increase the loading rate of the active ingredients and to adjust the control-release of the active ingredients. The filler can aid in the production or process of coating the seed. The amount of filler can vary, but generally the weight of the filler components will be in the range of about 0.05 to about 75% of the seed weight, more preferably about 0.1 to about 50%, and even more preferably about 0.5% to 15%.

The pesticides that are useful in the coating are those combinations of pesticides that are described herein. The amount of pesticide that is included in the coating will vary depending upon the type of seed and the type of active ingredients, but the coating will contain an amount of the combination of pesticides that is pesticidally effective. When insects are the target pest, that amount will be an amount of the combination of insecticides that is insecticidally effective.

As used herein, an insecticidally effective amount means that amount of insecticide that will kill insect pests in the larvae or pupal state of growth, or will consistently reduce or retard the amount of damage produced by insect pests. In general, the amount of pesticide in the coating will range from about 0.005 to about 50% of the weight of the seed. A more preferred range for the pesticide is from about 0.01 to about 40%; more preferred is from about 0.05 to about 20%.

The exact amount of the combination of pesticides that is included in the coating is easily determined by one of skill in the art and

will vary depending upon the size of the seed to be coated. The pesticides of the coating must not inhibit germination of the seed and should be efficacious in protecting the seed and/or the plant during that time in the target insect's life cycle in which it causes injury to the seed or plant. In general, the coating will be efficacious for approximately 0 to 120 days after sowing.

The coating is particularly effective in accommodating high pesticidal loads, as can be required to treat typically refractory pests, such as corn root worm, while at the same time preventing unacceptable phytotoxicity due to the increased pesticidal load.

Optionally, a plasticizer can be used in the coating formulation. Plasticizers are typically used to make the film that is formed by the coating layer more flexible, to improve adhesion and spreadability, and to improve the speed of processing. Improved film flexibility is important to minimize chipping, breakage or flaking during storage, handling or sowing processes. Many plasticizers may be used, however, useful plasticizers include polyethylene glycol, glycerol, butylbenzylphthalate, glycol benzoates and related compounds. The range of plasticizer in the coating layer will be in the range of from about 0.1 to about 20% by weight.

When the combination of pesticides used in the coating is an oily type formulation and little or no filler is present, it may be useful to hasten the drying process by drying the formulation. This optional step may be accomplished by means well known in the art and can include the addition of calcium carbonate, kaolin or bentonite clay, perlite, diatomaceous earth, or any absorbent material that is added preferably concurrently with the pesticidal coating layer to absorb the oil or excess moisture. The amount of calcium carbonate or related compounds necessary to effectively provide a dry coating will be in the range of about 0.5 to about 10% of the weight of the seed.

The coatings formed with the combination of pesticides are capable of effecting a slow rate of release of the pesticide by diffusion or movement through the matrix to the surrounding medium.

The coating can be applied to almost any crop seed that is described herein, including cereals, vegetables, ornamentals and fruits.

5 In addition to the coating layer, the seed may be treated with one or more of the following ingredients: other pesticides including fungicides and herbicides; herbicidal safeners; fertilizers and/or biocontrol agents. These ingredients may be added as a separate layer or alternatively may be added in the pesticidal coating layer.

10 The pesticide formulation may be applied to the seeds using conventional coating techniques and machines, such as fluidized bed techniques, the roller mill method, rotostatic seed treaters, and drum coaters. Other methods, such as spouted beds may also be useful. The seeds may be presized before coating. After coating, the seeds are typically dried and then transferred to a sizing machine for sizing. Such procedures are known in the art.

15 The pesticide-treated seeds may also be enveloped with a film overcoating to protect the pesticide coating. Such overcoatings are known in the art and may be applied using conventional fluidized bed and drum film coating techniques.

20 In another embodiment of the present invention, a pesticide can be introduced onto or into a seed by use of solid matrix priming. For example, a quantity of the pesticide can be mixed with a solid matrix material and then the seed can be placed into contact with the solid matrix material for a period to allow the pesticide to be introduced to the seed. The seed can then optionally be separated from the solid
25 matrix material and stored or used, or the mixture of solid matrix material plus seed can be stored or planted directly. Solid matrix materials which are useful in the present invention include polyacrylamide, starch, clay, silica, alumina, soil, sand, polyurea, polyacrylate, or any other material capable of absorbing or adsorbing
30 the pesticide for a time and releasing that pesticide into or onto the seed. It is useful to make sure that the pesticide and the solid matrix material are compatible with each other. For example, the solid matrix

material should be chosen so that it can release the pesticide at a reasonable rate, for example over a period of minutes, hours, or days.

5 The present invention further embodies imbibition as another method of treating seed with the pesticide. For example, plant seed can be combined for a period of time with a solution comprising from about 1% by weight to about 75% by weight of the pesticide in a solvent such as water. Preferably the concentration of the solution is from about 5% by weight to about 50% by weight, more preferably from about 10% by weight to about 25% by weight. During the period that
10 the seed is combined with the solution, the seed takes up (imbibes) a portion of the pesticide. Optionally, the mixture of plant seed and solution can be agitated, for example by shaking, rolling, tumbling, or other means. After imbibition, the seed can be separated from the solution and optionally dried, for example by patting or air drying.

15 In yet another embodiment, a powdered pesticide can be mixed directly with seed. Optionally, a sticking agent can be used to adhere the powder to the seed surface. For example, a quantity of seed can be mixed with a sticking agent and optionally agitated to encourage uniform coating of the seed with the sticking agent. The seed coated
20 with the sticking agent can then be mixed with the powdered pesticide. The mixture can be agitated, for example by tumbling, to encourage contact of the sticking agent with the powdered pesticide, thereby causing the powdered pesticide to stick to the seed.

25 The present invention also provides a seed that has been treated by the method described above.

30 The treated seeds of the present invention can be used for the propagation of plants in the same manner as conventional treated seed. The treated seeds can be stored, handled, sowed and tilled in the same manner as any other pesticide treated seed. Appropriate safety measures should be taken to limit contact of the treated seed with humans, food or feed materials, water and birds and wild or domestic animals.

Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is
5 intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples.

REFERENCE EXAMPLE 1

This example compares the efficacy of seed treatment with
10 lambda-cyhalothrin (CAS# 91465-08-6) to soil granular treatments with tefluthrin (CAS # 79538-32-2) against feeding damage by black cutworm larvae on shoots and foliage.

A lambda-cyhalothrin seed treatment formulation was prepared by diluting the WARRIOR® T insecticide (Zeneca Ag Products,
15 Wilmington, DE), which contains 11.4% lambda-cyhalothrin as the active ingredient, into water as a carrier. This formulation was applied for one minute at room temperature to twenty-five grams of Pioneer corn seed (Cultivar PN3394) in a rotostatic seed treater at a rate of 125 g, 250 g or 500 g active ingredient (AI) to 100 kg seed. The treated
20 seeds were allowed to sit uncapped for four to twenty-four hours before planting.

Treated and untreated seeds (Pioneer hybrid PN3394) were planted in a soil mix consisting of Dupo silt loam, 30% Perlite, 20% coarse sand (WB-10 grade) in six groups of tubs (20 in. L X 15 in. W X
25 8 in. D). Twelve seeds were planted per tub and three tubs were planted for each treatment regimen. Soil applications of FORCE® 3GR, which contains 3% tefluthrin granule as the active ingredient, were used for two sets of tubs containing untreated seeds. The FORCE 3GR was applied either in-furrow or incorporated into a 5 inch
30 band on the soil surface at the time of planting. The tubs were overhead irrigated until the plants were infested with black cutworm larvae.

The rate of application for the FORCE 3GR was reported in units of grams of the active ingredient per hectare (g/ha), while the rate of application of the WARRIOR T to the seeds was reported in units of grams of the active ingredient per 100 kilograms of the seeds (g/100 kg). Although the conversion of one of these units to the other will vary somewhat according to the type of seed that is being used, the size and weight of the seed, and the density of planting that is used – among other things – an approximate conversion for corn seed can be carried out as follows. Assuming a seed application rate of lambda cyhalothrin of, for example, 125g/100 kg of seed and a planting density of 15 lbs seed/ac, about 14.7 acres can be planted with 100 kg of the seed. This is an effective application rate of about 8.5g of lambda cyhalothrin per acre. At 2.47 ac/ha, the seed treatment level of 125g/100 kg is approximately equivalent to a surface banding treatment at about 21g/ha.

At twelve days after planting (DAP) but before infestation, the overall health of each plant was rated by looking at emergence, height and appearance. This vigor rating gives an indication of any phytotoxicity from the seed or soil treatment. A rating of 1 indicates extremely low vigor while 10 is the highest vigor rating.

The corn plants were infested at 12 DAP, which corresponds to late growth stage V1 by placing two black cutworm larvae at 3/4 instar on the soil surface near the base of the plant. Plants were rated 3, 7 and 10 days after infestation (DAI) for the number of cut plants, as well as damage from leaf feeding. The percent stand reduction due to plant cutting was calculated by dividing the number of cut plants into the number of plants present at infestation. The foliar feeding injury was evaluated using a rating scale of 1 = no damage and 10 = complete defoliation. The mean results for the three tubs for each treatment regimen are presented in Table 2 below.

Table 2. Efficacy of lambda-cyhalothrin seed-treatment against black cutworm feeding damage on corn.

Treatment Regimen	Vigor at 12 DAP	% Stand Reduct'n 3 DAI	Plant Defol. 3 DAI	% Stand Reduct'n 7 DAI	Plant Defol. 7 DAI	% Stand Reduct'n 10 DAI	Plant Defol. 10 DAI
None	8.0	72.8	9.0	94.4	9.3	100.0	10.0
λ -cyhalothrin seed 125 g/100 kg	9.0	13.9	4.3	16.7	5.0	19.4	3.3
λ -cyhalothrin seed 250 g/100 kg	8.3	3.0	3.7	3.0	2.7	3.0	1.7
λ -cyhalothrin seed 500 g/100 kg	8.3	0.0	2.0	0.0	2.3	0.0	1.0
Tefluthrin in-furrow 30 g/ha	9.0	33.9	5.0	48.0	6.0	48.0	5.3
Tefluthrin banded 30 g/ha	8.7	0.0	1.7	0.0	1.7	0.0	0.3

These results demonstrate that seed treatment with lambda-cyhalothrin prior to planting provides significant protection of corn plants against shoot/foiar feeding damage by black cutworm. For example, at 7 DAI with the lowest rate tested (125 g/kg seed), a significant reduction was observed for both plant cutting (16.7% for seed treatment vs. 94% for untreated control) and foliar feeding injury (5.0 for seed treatment vs. 9.3 rating for untreated control). In addition, tubs planted with seed treated with lambda-cyhalothrin at rates of 250 and 500 g/100 kg seed, showed essentially no stand reduction from plant cutting (3% and 0% for 250 and 500 g, respectively) and only low levels of foliar injury (2.7 and 2.3 rating for 250 and 500 g, respectively). This level of protection was equal to the tefluthrin soil band treatment and superior to tefluthrin in-furrow treatment. When the tubs were evaluated at 10 DAI, no increase in plant cutting and only

slightly higher ratings for foliar feeding injury were observed with lambda-cyhalothrin seed treatments as compared to evaluations at 7 DAI. In contrast, the untreated control tubs exhibited 100% plant cutting and complete defoliation by 10 DAI.

5

EXAMPLE 2

This example illustrates the efficacy of corn seed treatment with a combination of tefluthrin and thiamethoxam against plant damage by black cutworm.

Seed treatment formulations were prepared from tefluthrin (available under the trade name of RAZE® 2.5 FS, from Wilbur Ellis Co.) and thiamethoxam (3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-N-nitro-4H-1,3,5-oxadiazine-4-imine; CAS Registry No. 153719-23-4). In addition, separate seed treatment formulations were prepared from each of the two insecticides alone. These formulations were applied for one minute at room temperature to twenty-five grams of Pioneer corn seed (Cultivar PN3394) in a rotostatic seed treater at the rates shown in Table 3. The treated seeds were allowed to sit uncapped for four to twenty-four hours before planting.

Treated and untreated seeds (Pioneer hybrid PN3394) were planted in a soil mix consisting of Dupo silt loam, 30% Perlite, 20% coarse sand (WB-10 grade) in six groups of tubs (20 in. L X 15 in. W X 8 in. D). Twelve seeds were planted per tub and three tubs were planted for each treatment regimen.

The corn plants were infested at 12 days after planting (DAP), which corresponds to late growth stage V1 by placing two black cutworm larvae at 3/4 instar on the soil surface near the base of the plant. Plants were rated 10 days after infestation (DAI) for the number of cut plants, as well as damage from leaf feeding. The percent stand reduction due to plant cutting was calculated by dividing the number of cut plants into the number of plants present at infestation. The foliar feeding injury was evaluated using a rating scale of 1 = no damage and 10 = complete defoliation. The mean results for the three tubs for each treatment regimen are presented in Table 3 below.

Whether a combination of insecticides provided synergy in protection against cutworm damage was calculated as described by Colby, Robert. S., in *Weeds*, 15(1):20 - 22 (1967). The threshold value (stated as % of control) for synergy of a combination was calculated as

5 = $(\% \text{ of control for treatment A}) * (\% \text{ of control for treatment B}) / 100(n-1)$;

where n = number of active ingredients in combination. A measured % of control value that is less than the threshold value indicates synergy of the combination.

Threshold values for synergy were calculated for each

10 combination shown in Table 3 by the method described above. Threshold values for synergy are shown in Table 4.

Table 3: Protection of corn plants against black cutworm damage by seed treatments with tefluthrin, thiamethoxam and

15 combinations of the two.

TREATMENT	Tefluthrin (gm/100 kg seed)	Thiamethoxam (gm/100 kg seed)	STAND REDUCTION (% at 10 days)	Percent of Control	Synergy
RAZE	100		57.6	57.6	
RAZE	200		58.9	58.9	
RAZE	300		90.6	90.6	
ADAGE		200	96.9	96.9	
ADAGE		300	100	100	
RAZE/AD	100	200	58.9	58.9	NO
RAZE/AD	200	200	56.3	56.3	NO
RAZE/AD	300	200	60.3	60.3	NO
RAZE/AD	100	300	51.3	51.3	YES
RAZE/AD	200	300	37.5	37.7	YES
RAZE/AD	300	300	28.1	28.1	YES
UNTREATED CONTROL	0	0	100		

Table 4: Matrix of threshold values for synergy of combination
(% of control)

	RAZE @ 100	RAZE @ 200	RAZE @ 300
ADAGE @ 200	52.2	55.8	57.6
ADAGE @ 300	53.4	57.1	58.9

5 The results of this test showed that the combinations of tefluthrin and thiamethoxam were effective, and, in fact, were synergistic against damage to the plant by black cutworm for all levels of tefluthrin when levels of thiamethoxam were 300 gm/100 kg of seed (or about 0.3% by weight of the seed).

10 In view of the above, it will be seen that the several advantages of the invention are achieved and other advantageous results attained.

As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

15 The discussion of references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinency of the cited references.

WHAT IS CLAIMED IS:

1. A method for preventing damage by a pest to a seed and/or shoots and foliage of a plant grown from the seed, the method comprising treating the unsown seed with a composition comprising thiamethoxam and at least one pyrethrin or synthetic pyrethroid.
5
2. The method as set forth in claim 1, wherein the pyrethrin or synthetic pyrethroid is selected from the group consisting of taufluvallinate, flumethrin, trans-cyfluthrin, kadethrin, bioresmethrin, tetramethrin, phenothrin, empenethrin, cyphenothrin, prallethrin,
10 imiprothrin, allethrin and bioallethrin.
3. The method as set forth in claim 1, wherein the composition is included in a seed coating.
4. The method as set forth in claim 3, wherein at least one of the pyrethroids is a systemic insecticide.
- 15 5. The method as set forth in claim 3, wherein the seed is treated with the composition of the pyrethroid and the thiamethoxam in an amount effective to provide protection to the seed and/or the shoots and foliage of the plant against damage by the pest.
6. The method as set forth in claim 3, wherein the seed is
20 treated with the pyrethroid at the same time that it is treated with the thiamethoxam.
7. The method as set forth in claim 3, wherein the seed is treated with the pyrethroid as a different time than it is treated with the thiamethoxam.
- 25 8. The method as set forth in claim 3, wherein the composition is included in a seed coating.
9. A seed that has been treated by the method as set forth in claim 1.
10. The seed as set forth in claim 9, wherein the seed is
30 selected from the group consisting of corn, soybean, cotton, rice, sorghum, sugar beet, wheat, barley, rye, sunflower, tomato, sugarcane, tobacco, rape and oats.

11. The seed as set forth in claim 10, wherein the seed is selected from the group consisting of corn, soybean and cotton seed.

12. The seed as set forth in claim 11, wherein the seed is corn seed.

5 13. The seed as set forth in claim 10, wherein the seed is a transgenic seed.

14. A composition for the treatment of unsown seed comprising thiamethoxam and at least one pyrethrin or synthetic pyrethroid is selected from the group consisting of taufluvalinate,
10 flumethrin, trans-cyfluthrin, kadethrin, bioresmethrin, tetramethrin, phenothrin, empenethrin, cyphenothrin, prallethrin, imiprothrin, allethrin and bioallethrin.

15. A seed that is protected against multiple pests comprising a seed having at least one heterologous gene encoding for the
15 expression of a protein that is active against a first pest and, in addition, having adhered thereto a composition comprising at least one pyrethrin or synthetic pyrethroid and thiamethoxam, where the composition is present in an amount effective to provide protection to the shoots and foliage of the plant against damage by at least one
20 second pest.

16. The seed as set forth in claim 15, wherein the at least one heterologous gene encodes for the expression of a protein that is insecticidally active.

17. The seed as set forth in claim 16, wherein the gene is one
25 originally derived from a microorganism selected from the group consisting of *Bacillus*, *Rhizobium*, *Pseudomonas*, *Serratia*, *Trichoderma*, *Glomus*, *Gliocladium* and mycorrhizal fungi.

18. The seed as set forth in claim 17, wherein the protein is active against corn root worm.

30 19. The seed as set forth in claim 17, wherein the protein is active against european corn borer.

20. The seed as set forth in claim 19 wherein the gene is one originally derived from a *Bacillus sp.* microorganism.

21. The seed as set forth in claim 20, wherein the gene is one originally derived from *Bacillus thuringiensis*.

22. The seed as set forth in claim 21, wherein the gene is one that encodes for the production of a modified Cry3Bb delta-endotoxin.

23. The seed as set forth in claim 15, wherein the seed is selected from the group consisting of corn, soybean, cotton, rice, sorghum, sugar beet, wheat, barley, rye, sunflower, tomato, sugarcane, tobacco, rape and oats.

24. A method for treating an unsown seed to prevent damage by a pest to the seed and/or shoots and foliage of a plant grown from the seed, the method comprising contacting the unsown seed with a composition comprising thiamethoxam and at least one pyrethrin or synthetic pyrethroid which is selected from the group consisting of taufluvalinate, flumethrin, trans-cyfluthrin, kadethrin, bioresmethrin, tetramethrin, phenothrin, empenthrin, cyphenothrin, prallethrin, imiprothrin, allethrin and bioallethrin.

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(54) Title: SEED TREATMENT WITH COMBINATIONS OF PYRETHRINS/PYRETHROIDS AND THIAMETHOXAM

(57) Abstract: A method of preventing damage to the seed and/or shoots and foliage of a plant by a pest includes treating the seed from which the plant grows with a composition that includes a combination of thiamethoxam and at least one pyrethrin or synthetic pyrethroid which is selected from the group consisting of taufluvallinate, flumethrin, trans-cyfluthrin, kadethrin, bioresmethrin, tetramethrin, phenothrin, empenethrin, cyphenothrin, prallethrin, imiprothrin, allethrin and bioallethrin. The treatment is applied to the unsown seed. In another embodiment, the seed is a transgenic seed having at least one heterologous gene encoding for the expression of a protein having pesticidal activity against a first pest and the composition has activity against at least one second pest. Treated seeds are also provided.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, EPO-Internal, CHEM ABS Data, CAB Data

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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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